

IN THE SPECIFICATION:

Please replace the paragraph on page 1, lines 3-4, with the following rewritten paragraph:

This filing is a divisional of commonly assigned, co-pending application 09/970,446, filed October 3, 2001, which patent application claims benefit of U.S. provisional patent application No. 60/239,023, filed October 4, 2000, each of which is incorporated by reference.

Please replace the paragraph on page 6, lines 9-31, with the following rewritten paragraph:

Glucocorticoids (GC), including Dexamethazone Dexamethasone (Dex), are potent antiinflammatory and immunosuppressive agents that are widely used in the treatment of inflammatory disorders, such as autoimmune and allergic diseases (Wilkens and de Wilckens and de De Rijk (1997) Immunol. Today 18:418-424; Schleimer, et al. (ed. 1997) (eds.) (1997) Inhaled Glucocorticoids in Asthma: Mechanisms & Clinical Actions, Marcel Dekker, NY, NY). GC have been shown to have an inhibitory effect on both T cells and APC, at the level of proliferation as well as cytokine production, with down-regulation of IFN- γ , IL-4, and IL-5 under some conditions, but upregulation of IL-4 under other conditions. See Blotta, et al. (1997) J. Immunol. 158:5589-5595; Ramirez, et al. (1996) J. Immunol. 156:2406-2412; and Daynes and Araneo (1989) Eur. J. Immunol. 19:2319-2325. This may result from indirect effects, e.g., GC down-regulate the production of IL-12 by APC and thus IFN- γ production by T cells (Blotta, et al. (1997) J. Immunol. 158:5589-5595; Vieira, et al. (1998) J. Immunol. 161:5245-5251; and Visser, et al. (1998) Blood 91:4255-4264), and thus in some cases may indirectly upregulate the production of IL-4 (Blotta, et al. (1997) J. Immunol. 158:5589-5595) and or IL-5 (Vieira, et al. (1998) J. Immunol. 161:5245-5251) and/or IL-10 (Vieira, et al. (1998) J. Immunol. 161:5245-5251; and Visser, et al. (1998) Blood 91:4255-4264) in cultures contain APC, antigen, and T cells. Recently, it has been shown that GC drive human CD8+ T cell differentiation towards a stable

phenotype with high IL-10 and reduced IFN- γ , IL-4, IL-5 and IL-13 production. See Richards and Hawrylowicz , et al. (2000) Eur. J. Immunol. 30:2344-2354.

Please replace the paragraph beginning on page 6, line 32, and continuing to page 7, line 12, with the following rewritten paragraph:

GC bind the cytosolic GC receptor (GR), which then translocates to the nucleus and inhibits the transcriptional activation of target genes. See review of Karin, in Schleimer, et al. (ed. 1997) supra Inhaled Glucocorticoids in Asthma: Mechanisms & Clinical Actions Dekker; and Karin (1998) Cell 93:487-490. GC mediate transcriptional repression through: 1) interfering with the function of transacting factors, such as AP-1 and NF κ B (De Bosscher, et al. (1997) Proc. Nat'l. Acad. Sci. USA 94:13504-13509), via protein-protein interactions; and inhibition of NFAT binding to cytokine gene promoters (Chen, et al. (2000) J. Immunol. 164:825-832); 2) direct DNA binding to poorly conserved negative GC responsive elements (GRE); or 3) inducing the expression of inhibitory factors such as IkBa (reviewed in Karin in Schleimer, et al., supra (eds. 1997) Inhaled Glucocorticoids in Asthma: Mechanisms & Clinical Actions Dekker). Furthermore, GR represses TGF- β transcriptional activation of the plasminogen activator PAI-1 gene in a ligand-dependent manner, by both Smad3 and Smad4 C-terminal activation domains. See Song, et al. (1999) Proc. Nat'l Acad. Sci. USA 96:11776-11781.

Please replace the paragraph beginning on page 7, line 28, and continuing to page 8, line 10, with the following rewritten paragraph:

1,25(OH)-dihydroxyvitamin D3 1,25(OH)-Dihydroxyvitamin D3 (VitD3) is a secosteroid receptor hormone that binds to a nuclear receptor named Vitamin D3 receptor (VDR). Once bound to the hormone ligand the receptor associates with specific recognition sequences called vitamin D responsive elements (VDRE) which are present in the promoter regions of target genes and are involved in regulating their transcription. Recent studies have shown that VitD3 represses IL-2 gene transcription by VDR-dependent inhibition of NFATp/AP-1 complex formation. Alroy, et al. (1995)

Mol. Cell Biol. 15:5789-5799. In addition, it has also been reported that VitD3 mediates downregulation of NF-kB activation by decreasing NF-kB p50 and c-Rel expression in T cells. Yu, et al. (1995) Proc. Nat'l Acad. Sci. USA 92:10990-10994. The inhibition of transcription activation of the IL-12 p35 and p40 genes by VitD3 may be, in part, by downregulation of NF-kB activation and binding to the p40-kB sequence. D'Ambrosio, et al. (1998) J. Clin. Invest. 101:252-262. In addition, SMAD3, one of the SMAD proteins downstream in the TGF- β signaling pathway, which was inhibited by GC, acts as a coactivator specific for ligand-induced transactivation of VDR. Yanagisawa, Yanagisawa, et al. (1999) Science 286:1317-1321.

Please replace the paragraph on page 12, lines 17-26, with the following rewritten paragraph:

Stimulation of naïve naive T cells (CD4+CD62L+) using Antigen Presenting Cells (APC) in the presence of VIT D3 and DEX gives rise to: about 60% IL-10 positive cells (producing at least 500 ng of IL-10 per 10⁶ cells); about 10% IL-10 and IL-4 double positive cells (producing about 50 ng of IL-4 per 10⁶ cells); less than about 1% IL-5 positive cells (producing less than about 30 pg of IL-5 per 10⁶ cells); and less than about 5% IFN- γ positive cells (producing less than about 40 ng of IFN- γ per 10⁶ cells). The cytokine profiles produced by the cells after restimulation are evaluated after 6 h by FACS analysis, while the quantities are accumulated by the respective cell population in 1 ml for 48 h.

Please replace the paragraph on page 12, lines 27-35, with the following rewritten paragraph:

Alternatively, stimulation of naïve naive T cells using Antigen Presenting Cells (APC) in the presence of VitD3 and Dex + anti-IL-4 + anti-IL-12 + anti-IFN- γ gives rise to a population of cells: about 25-35% IL-10 positive cells (producing at least about 100 ng of IL-10 per 10⁶ cells); less than about 1% IL-4 positive cells (producing about 30-1000 pg of IL-4 per 10⁶ cells); less than about 1% IL-5 positive cells (producing less than about 30 pg of IL-5 per 10⁶ cells); less than about 1% IFN- γ positive cells (producing less than about 30 pg of IFN- γ per 10⁶ cells); and less than about 5% IL-2 positive cells (producing about 30-350 pg IL-2 per 10⁶ cells).

Please replace the paragraph on page 13, lines 1-7, with the following rewritten paragraph:

If the stimulation of naïve naive T cells is performed in the presence of anti-CD3 + anti-CD28 with VitD3 and Dex, the resulting population of cells is: about 70-75% IL-10 positive cells; less than about 2% IL-4 positive cells; less than about 1% IL-5 positive cells; less than about 1% IFN- γ positive cells; and less than about 2% IL-2 positive cells. Corresponding quantities of cytokines should be detected according to the cell types described immediately above.

Please replace the paragraph on page 13, lines 8-12, with the following rewritten paragraph:

Stimulation of naïve naive T cells using anti-CD3 + anti-CD28 in the presence of VitD3 and Dex + anti-IL-4 + anti-IL-12 + anti-IFN- γ gives rise to a population of: about 60% IL-10 positive cells; less than about 1% IL-4 positive cells; less than about 1% IL-5 positive cells; less than about 1% IFN- γ positive cells; and about 10% IL-2 and IL-10 double positive cells.

Please replace the paragraph on page 13, lines 13-24, with the following rewritten paragraph:

This data on the development of IL-10 only producing cells using Vitamin D3 and Dexamethazone plus antigenic stimulation of CD4+ T cells has also been reproduced in human systems. In this case there is some dependency on IL-4. Stimulation of purified human naïve naive T cells from cord-blood (CD4+CD45RA+) using L cells (expressing CD32, CD86) as APC plus anti-CD3 in the presence of VitD3 and Dex gives rise after one week of stimulation to a majority of IL-10 producing T cells: VitD3 is used at 2×10^{-8} M (2×10^{-9} M to 2×10^{-7} M) while Dex is used at 10^{-7} M (10^{-8} M to 10^{-6} M). In addition, stimulation of purified naïve naive T cells enriched from human peripheral blood (CD4+CD45RA+) using anti-CD3/+- anti-CD28, in the presence of VitD3 and Dex gives rise after one week of stimulation to a majority of IL-10 producing T cells.

Please replace the paragraph beginning on page 24, line 30, and continuing to page 25, page 16, with the following rewritten paragraph:

Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood, freshly collected into sodium citrate, by centrifugation on a Lymphoprep ® (Nycomed, Birmingham, UK) density gradient. Donors were healthy at the time of the study. PBMC were positively selected for CD8+ or CD4+ T cells using antibody-coated magnetic beads and Detach-a-beads ® (Dynal (UK), Wirral, GB) according to the manufacturer's guidelines. Alternatively, PBMC were depleted of CD4+ or CD8+ T cells by negative selection using antibody-conjugated magnetic beads to give CD8+ APC or CD4+ APC populations, respectively. CD4+ T cells were further subdivided into naïve naive CD45RA+ and antigen-experienced CD45RO+ populations using specific antibodies (PharMingen, San Diego, CA) and negative selection with magnetic beads. APC consisted predominantly of CD4low, CD14+ monocytes (13-28%), although a minor population (1.3-5%) of CD4low, CD14- cells is likely to contain DC. Isolated populations were washed and resuspended at 1×10^6 /ml in RPMI 1640 (Life Sciences, Abingdon, GB) containing 10% heat-inactivated FCS fetal calf serum (FCS) (PAA Laboratories, Oxford, GB), 2 mM L-glutamine, and 50 µg/ml gentamycin (both from Life Sciences). Cell purity was assessed by flow cytometry using a FACScan ® (Becton

Dickinson, Abingdon, GB) and Lysis 2 software. Anti-CD4 (Leu-3a) and CD14 (Leu-M3) antibodies were purchased from Becton Dickinson; CD3 (UCHT1), CD8 (UCHT4), isotype-matched control IgG1 (MOPC21), and IgG2b (MOPC141) antibodies were from Sigma (Poole, GB).